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THE

# SOURCE AND NATURE OF BACTERIA IN MILK.

BY

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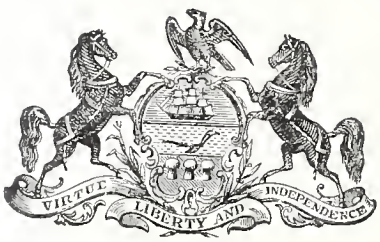
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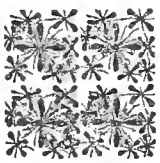


# PREFACE.

Harrisburg, Pa., July 2, 1904.

The greatest developments in modern dairy practice have been in relation to the study and control of the bacteria of milk. When it was shown that souring of milk is caused by the growth of bacteria, which transform milk sugar into lactic acid, it became evident that the control of the keeping properties of milk depends upon the control of these diminutive plants. Cleanliness, sterilization, cooling, cold-storage and artificial preservatives, all have for their object the control of bacteria.

It is now known not only that the life of milk, but also its wholesomeness. depend upon its freedom from harmful bacteria. Hence, it is the purpose of the most progressive dairymen to so conduct the business of milk production and distribution, as, first, to keep at a minimum the number of bacteria gaining admission to milk, and, second, so far as possible to prevent the growth of those that do gain entrance.

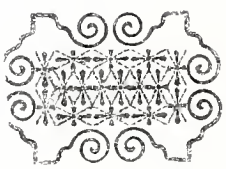
In recent years the business of producing milk as free as possible from bacteria has reached large proportions, and such milk sells for a price considerably in advance of the price of ordinary market milk.

If the demand for especially "clean milk" continues to grow, as seems assured, information in relation to the principles underlying this form of milk production will increase in importance.

This Bulletin is prepared by an expert bacteriologist, who is entirely familiar with Pennsylvania dairy-farm conditions. It is devoted chiefly to a study of the principles underlying the production of milk containing few bacteria, but it also takes up the question of the origin and the frequency of pus cells in milk, a subject of much importance.

It is hoped that this Bulletin will furnish needed information not only to producers of the highest class of market milk, but also to dairymen who wish to improve the keeping qualities, the wholesomeness and the salability of their product.

N. B. CRITCHFIELD,
Secretary of Agriculture.



# LETTER OF TRANSMITTAL.

Department of Agriculture,
Dairy and Food Division,
Harrisburg, Pa., July 1, 1904.

To Hon. N. B. Critchfield, *Secretary of Agriculture, Harrisburg, Pa.:*

Sir: I have the honor to hand you herewith the manuscript of a report on an investigation upon the source and nature of bacteria in milk by Dr. D. H. Bergey.

I requested the preparation of this report, because the subject is one of great importance to progressive dairymen, and is so new in many of its developments that it is difficult to obtain information upon it.

The investigation upon which the report is based was conducted in the Laboratory of Hygiene, University of Pennsylvania, during the months of June, July, August and September, 1903.

I beg to recommend that it be published as a bulletin of the Department of Agriculture.

Respectfully yours,

B. H. WARREN,
Commissioner.

# SOURCE AND NATURE OF BACTERIA IN MILK.

## OUTLINE OF THE INVESTIGATION.

This investigation is made with special reference to the following points:

1. The number and nature of the bacteria in milk as it comes from the udder.

2. The nature and source of the bacteria gaining access to the milk in the ordinary manipulations in collecting and storing milk in modern dairies and milkhouses.

3. The occurrence of leucocytes and pus cells in the milk of healthy cows, and a determination of the significance of their presence in the milk.

4. The prevalence of streptococci in milk and a determination of the relation of these bacteria to inflammatory conditions of the udder.

5. The relation of the streptococci found in milk to those encountered in scarlet fever, diphtheria, rheumatism, erysipelas, and other diseases of man and the domestic animals.

## MANNER OF CONDUCTING THE INVESTIGATION.

1. The study of the number and nature of the bacteria in freshly drawn milk, is to be carried out by plating definite volumes of samples of milk collected in sterile flasks with the greatest possible care to avoid outside contamination. By counting the colonies developing in such plates, the number of bacteria in a definite volume of the milk will be ascertained. By subsequent study of the bacteria developing in these plates upon different culture media, their nature will be determined.

2. The nature and source of the bacteria gaining access to the milk in the ordinary manipulations, is to be determined by plating samples collected from the pails, strainer, cooler, etc., and noting the increase in the number of bacteria arising from these sources, and from the air of the stable, the fur of the animal, and the hands and clothing of the milker.

3. The occurrence of leucocytes and pus cells in the milk is to be determined by centrifuging the samples of milk and staining a drop of the sediment. The determination of the significance of leucocytes and pus cells in milk is to be carried out by studying the

milk of healthy cows as well as of cows presenting inflammatory disease of the udder, and also by examining the milk of several cows over a period of months in order to determine the influence of food, weather, hygiene of the surroundings, oestrum, period of lactation, etc., upon the occurrence and prevalence of such cells.

4. The study of the prevalence of streptococci in milk is to be made by staining the sediment obtained by centrifuging the milk and by isolating the streptococci from the plate cultures of the different samples of milk. The determination of the relation of the streptococci to inflammatory conditions of the udder is to be made by careful physical examination of the udders of cows whose milk shows the presence of streptococci, as well as by ascertaining the prevalence of these bacteria in the milk of these cows by observations and microscopic studies conducted through a period of several months.

5. The study of the relation of the streptococci found in milk to other varieties of streptococci encountered in diseases of man and the domestic animals, is to be made by the systematic study and comparison of these bacteria in different culture media, and by the immunization of goats or calves against the streptococci found in milk, and testing the blood serum of the immune animals upon different races of streptococci according to the method employed by Aronson in his studies upon streptococci.

Eight modern dairies were visited and samples of milk collected in sterile test tubes from individual cows, and from the milk pails and cooling apparatus. The samples were at once packed in ice and brought to the laboratory and analyzed the next morning. Samples were collected from individual cows by milking into the test tube, and frequently a separate sample was taken from each quarter of the udder. Samples were also collected from the milk buckets and from the straining, cooling and bottling apparatus. Frequently the milk of one or two cows was sampled in this way in its progress from the udder to the final container so as to determine the points at which bacteria gained access to the milk. In several instances samples were taken of the first milk passing over the straining, cooling, and bottling apparatus, and after from 50 to 100 quarts of milk had passed over this apparatus, another set of samples was collected in order to determine whether the number of bacteria gaining access to the milk at the different points remained the same.

The samples of milk collected directly from the udder were diluted with nine parts of sterile distilled water, while the samples collected from the milking and cooling utensils were diluted with nineteen parts of sterile distilled water before plating.

Of the milk diluted in the above manner, two portions, of 1-10 and

1-5 of a cubic centimeter, respectively, were mixed in each of two tubes of sterile neutral agar medium and the contents of each tube poured out into a sterile glass-covered dish (Petri plate), and when the agar had solidified, the plates were placed in an incubator kept at about the body temperature, 36 degrees to 37 degrees (Centigrade.)

The number of colonies of bacteria developing on the plates was enumerated after 24 and 48 hours, and where the counts could be made satisfactorily at 48 hours, the results obtained at that time were used in estimating the number of bacteria in the milk.

After a small portion of each sample of milk, collected on a particular day, had been diluted and plated as described, 10 cubic centimeters of each sample were placed in a centrifuge tube and centrifugalized for ten minutes. By this means the leucocytes and pus cells in the milk were collected in the bottom of the centrifuge tube. This sediment was then spread in a thin layer on clean coverslips, dried and fixed in the flame, treated with chloroform to remove the fat, and stained with Loeffler's alkaline methylene blue solution. These preparations were then examined microscopically with the 1-12 immersion lens and the number of cells in 10 fields of the microscope enumerated.

## RESULTS OF THE INVESTIGATION.

*1. The number and nature of the bacteria in milk as it comes from the udder.*

Table I shows the results obtained in the analysis of samples of milk collected directly from the udder in sterile test tubes.

TABLE I.

Results obtained with milk drawn directly from the udder and collected in sterile test tubes:

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 1 | 100 | 45. | Staphylococcus citreus. | |
| 6 | 0 | 0.5 | | |
| 7 | 200 | 0.05 | Bact. lactis. | |
| 8 | 0 | 0.1 | | |
| 9 | 11,425 | 40. | Streptococcus pyogenes. | |
| 10 | 150 | 8. | Staphylococcus albus. | |
| 11 | 25 | 0.05 | Staphylococcus aureus. | |
| 12 | 25 | 8. | Staphylococcus aureus. | |
| 13 | 0 | 0.05 | | |
| 14 | 450 | 2. | Staphylococcus albus. | |
| 15 | 1,075 | 0 | Staphylococcus albus.<br>B. pseudodiphtheria. | |

TABLE I—Continued.

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 16 | 25 | 0 | (Culture lost.) | |
| 17 | 1,300 | 1. | Streptococcus pyogenes. | |
| 18 | 200 | 0.05 | Sarcina alba.<br>Staphylococcus albus. | |
| 19 | 150 | 0.1 | Staphylococcus aureus. | |
| 20 | 0 | 0.5 | | |
| 21 | 100 | 0 | Bact. solare.<br>Staphylococcus albus. | |
| 22 | 0 | 0.1 | | |
| 23 | 200 | 0.05 | Staphylococcus aureus. | |
| 24 | 100 | 5. | (Culture lost.) | |
| 25 | 0 | 0.2 | | |
| 26 | 50 | 5. | Staphylococcus albus. | |
| 27 | 50 | 0.05 | Staphylococcus citreus.<br>B. pseudodiphtheria. | |
| 28 | 0 | 25. | | |
| 29 | 25 | 0.1 | M. acidi lactici. | |
| 33 | 50 | 0.04 | | |
| 34 | 100 | 15. | Staphylococcus albus. | |
| 35 | 850 | 1. | Staphylococcus albus. | |
| 36 | 45 | 0.04 | B. matazoonii.<br>B. pseudodiphtheria. | |
| 37 | 100 | 0.02 | Staphylococcus citreus. | |
| 38 | 1,000 | 0 | Staphylococcus citreus. | |
| 39 | 5,325 | 0 | Staphylococcus aureus. | |
| 40 | 0 | 0 | | |
| 41 | 1,225 | 0.5 | Staphylococcus aureus. | |
| 42 | 125 | 45. | Staphylococcus aureus and citreus. | |
| 45 | 100 | 2. | (Culture lost.) | |
| 46 | 800 | 1. | B. pseudodiphtheria, ..... | Fresh. |
| 47 | 2,100 | 1. | Staphylococcus albus. | |
| 48 | 150 | 8. | Staphylococcus aureus. | |
| 49 | 300 | 0.02 | Staphylococcus citreus.<br>B. convolutum.<br>Bact. conni. | |
| 52 | 20,075 | 0.2 | Streptococcus pyogenes. | |
| 56 | 75 | 0.7 | (Culture lost.) | |
| 57 | 175 | 1.6 | Streptococcus pyogenes. | |
| 58 | 3,250 | 1.5 | Staphylococcus albus, ..... | Nearly dry. |
| 59 | 100 | 2.3 | B. pseudodiphtheria. | |
| 60 | 0 | 0 | ..... | Nearly dry. |
| 61 | 0 | 4.1 | ..... | Nearly fresh. |
| 62 | 0 | 0 | ..... | **Left hindquarter.** |
| 63 | 25 | 0.1 | B. stoloniferus<br>Staphylococcus citreus. | |
| 64 | 3,925 | 7.8 | Staphylococcus aureus. | |
| 65 | 0 | 0.1 | | |
| 67 | 8,800 | 1.0 | Streptococcus pyogenes, ..... | Aborted several weeks previous. |
| 68 | 100 | 2.3 | M. acidi lactici. | |
| 69 | 500 | 0.1 | Staphylococcus albus. | |
| 70 | 50 | 0 | Staphylococcus aureus. | |
| 71 | 25 | 0.5 | (Culture lost.) | |
| 72 | 0 | 2.0 | | |
| 73 | 700 | 100. | Sarcina alba.<br>B. pseudodiphtheria. | |
| 74 | 0 | 1.3 | | |
| 75 | 0 | 0.1 | | |
| 76 | 0 | 0.2 | | |
| 77 | 0 | 0 | | |

TABLE I—Continued.

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 78 | 0 | 2.8 | | |
| 81 | 0 | 0 | | |
| 85 | 0 | 0.8 | | |
| 86 | 0 | 8.1 | ..... | Nearly fresh. |
| 87 | 100 | 0.2 | (Culture lost), ..... | Nearly dry. |
| 88 | 875 | 19. | B. coli, ..... | Nearly dry. |
| 89 | 25 | 0.6 | Streptococcus pyogenes. | |
| 90 | 675 | 15.3 | Staphylococcus aureus. | |
| 91 | 150 | 1.3 | Staphylococcus aureus and albus.<br>Streptococcus pyogenes. | |
| 92 | 825 | 7.2 | Staphylococcus aureus. | |
| 93 | 125 | 2.9 | Staphylococcus citreus. | |
| 94 | 100 | 1.4 | Staphylococcus aureus. | |
| 95 | 75 | 0.3 | Staphylococcus albus. | |
| 96 | 4,325 | 6.5 | Staphylococcus albus.<br>B. théta. | |
| 97 | 10,475 | 50. | Streptococcus pyogenes. | |
| 98 | 25 | 1.1 | Staphylococcus aureus. | |
| 99 | 3,425 | 1.6 | Staphylococcus aureus.<br>B. pseudodiphtheria. | |
| 100 | 0 | 1.3 | | |
| 101 | 425 | 0.4 | Staphylococcus albus. | |
| 104 | 300 | 7.6 | Staphylococcus aureus. | |
| 105 | 75 | 0.2 | Staphylococcus citreus. | |
| 106 | 1,050 | 14.3 | Streptococcus pyogenes. | |
| 107 | 0 | 0.2 | | |
| 108 | 15,600 | 0.1 | Bact. fulvum. | |
| 109 | 775 | 10.2 | Streptococcus pyogenes. | |
| 110 | 150 | 3.1 | B. pseudodiphtheria, ..... | Nearly dry. |
| 111 | 25 | 0.1 | Staphylococcus albus, ..... | Nearly fresh. |
| 112 | 25 | 0.1 | Staphylococcus albus. | |
| 113 | 0 | 0.3 | | |
| 114 | 475 | 35. | B. pseudodiphtheria.<br>Streptococcus pyogenes. | |
| 115 | 100 | 12.4 | Staphylococcus albus.<br>Streptococcus pyogenes. | |
| 116 | 25 | 1.8 | Staphylococcus albus. | |
| 117 | 75 | 0 | (Culture lost.) | |
| 118 | 0 | 1.7 | | |
| 119 | 93,100 | 8.6 | Streptococcus pyogenes. | |
| 120 | 0 | 1.4 | | |
| 121 | 25 | 0 | Staphylococcus albus. | |
| 122 | 0 | 2.7 | | |
| 123 | 100 | 3.9 | Staphylococcus aureus. | |
| 124 | 0 | 0.2 | | |
| 125 | 325 | 0.3 | Staphylococcus aureus. | |
| 126 | 75 | 5.4 | Sarcina alba. | |
| 127 | 100 | 1.9 | Staphylococcus albus. | |
| 128 | 200 | 0.9 | M. tetragenus. | |
| 129 | 50 | 6.0 | Sarcina subflava. | |
| 130 | 325 | 0.7 | Staphylococcus albus. | |
| 131 | 500 | 51.7 | Staphylococcus albus. | |
| 132 | 0 | 0.2 | | |
| 133 | 200 | 0.4 | Streptococcus pyogenes. | |
| 134 | 0 | 0.7 | | |
| 135 | 5,100 | 3.8 | Streptococcus pyogenes. | |
| 136 | 0 | 25. | ..... | Fresh. |
| 137 | 100 | 0.5 | Staphylococcus albus. | |
| 138 | 200 | 21.7 | Bact. connii. | |

TABLE I—Continued.

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 139 | 75 | 0.1 | Cladothrix dichotoma. | |
| 140 | 275 | 0.2 | (Culture lost.) | |
| 141 | 125 | 4.3 | Staphylococcus aureus and albus. | |
| 142 | 13,950 | 8.0 | Streptococcus pyogenes. | |
| 143 | 6,425 | 50.+ | Streptococcus pyogenes.<br>M. tetragenus. | |
| 144 | 175 | 20.0 | Staphylococcus citreus. | |
| 145 | 1,700 | 25. | Streptococcus pyogenes. | |
| 146 | 0 | 0.1 | | |
| 147 | 0 | 0.4 | | |
| 148 | 775 | 12. | B. alkaligenes.<br>Streptococcus pyogenes. | |
| 149 | 750 | 0.4 | Staphylococcus albus. | |
| 150 | 0 | 0.5 | | |
| 151 | 0 | 0.4 | | |
| 152 | 50 | 2.2 | Staphylococcus albus. | |
| 153 | 275 | 4.5 | Staphylococcus albus. | |
| 154 | 125 | 50.+ | B. schafferi.<br>Staphylococcus albus. | |
| 155 | 0 | 0.6 | | |
| 156 | 0 | 9.5 | ..... | Fresh 8 days previous. |
| 157 | 350 | 7.1 | Bact. lactis, .....<br>B. pseudodiphtheria. | Fresh 5 days previous. |
| 158 | 3,550 | 50.+ | Streptococcus pyogenes.<br>B. pseudodiphtheria. | |
| 159 | 850 | 18. | Staphylococcus albus, .....<br>B. pseudodiphtheria. | Within about 5 days of calving. |
| 160 | 450 | 3. | Bacterium.<br>Staphylococcus albus. | |
| 161 | 0 | 0.5 | | |
| 162 | 75 | 1.6 | B. pseudodiphtheria. | |
| 163 | 0 | 0.7 | | |
| 164 | 100 | 6.4 | M. tetragenus. | |
| 165 | 0 | 1.2 | | |
| 166 | 400 | 15. | Bact. delta.<br>Staphylococcus aureus. | |
| 167 | 0 | 1. | | |
| 168 | 0 | 6.1 | | |
| 169 | 350 | 0.2 | B. pseudodiphtheria.<br>M. tetragenus. | |
| 170 | 100 | 4.2 | Staphylococcus aureus. | |
| 171 | 100 | 2.5 | Staphylococcus aureus. | |
| 172 | 25 | 50.+ | Staphylococcus albus. | |
| 173 | 0 | 0.1 | | |
| 174 | 0 | 0.1 | | |
| 175 | 34,725 | 50.+ | Streptococcus pyogenes. | |
| 176 | 0 | 5. | | |
| 177 | 0 | 0.8 | | |
| 178 | 0 | 0.3 | | |
| 179 | 0 | 5.9 | | |
| 180 | 2,075 | 50.+ | Streptococcus pyogenes. | |
| 181 | 0 | 0.2 | | |
| 182 | 25 | 1.4 | Staphylococcus citreus. | |
| 183 | 5,225 | 26.3 | Streptococcus pyogenes. | |
| 184 | 550 | 0.6 | Staphylococcus aureus. | |
| 185 | 0 | 8. | | |
| 186 | 0 | 0.1 | | |
| 187 | 100 | 0 | Bact. convolutum.<br>Diplococcus. | |

TABLE I—Continued:

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 188 | 600 | 25. | Staphylococcus aureus. | |
| 189 | 0 | 1.9 | | |
| 190 | 0 | 0.2 | | |
| 191 | 4,950 | 15 | Streptococcus pyogenes. | |
| 192 | 25 | 0.7 | B. stoloniferus. | |
| 193 | 0 | 1.5 | | |
| 194 | 4,025 | 50.+ | Streptococcus pyogenes. | |
| 195 | 0 | 1.4 | | |
| 196 | 0 | 4.9 | | |
| 198 | 0 | 0.7 | | |
| 199 | 0 | 1.8 | | |
| 201 | 0 | 2.5 | | |
| 202 | 0 | 0.5 | | |
| 203 | 3,050 | 7.0 | Staphylococcus albus. | |
| 204 | 0 | 5.5 | | |
| 205 | 575 | 25.+ | Staphylococcus albus. | |
| 206 | 0 | 6.3 | | |
| 207 | 0 | 0.9 | | |
| 208 | 150 | 10.2 | Staphylococcus albus. | |
| 209 | 2,200 | 35.+ | Streptococcus pyogenes. | |
| 210 | 75 | 1.1 | Staphylococcus albus. | |
| 219 | 67,050 | ..... | Streptococcus pyogenes, ..... | Contagious mammitis. |
| 220 | 0 | 3.1 | | |
| 221 | 0 | 3.2 | | |
| 222 | 0 | 25.+ | | |
| 223 | 0 | 1.4 | | |
| 224 | 25 | 10.1 | Staphylococcus albus. | |
| 225 | 75 | 0.1 | Sarcina alba. | |
| 226 | 0 | 3.1 | ..... | R. h. quarter. |
| 227 | 100 | 6.1 | (Culture lost), ..... | L. h. quarter. |
| 228 | 50 | 0.9 | Staphylococcus aureus, ..... | L. f. quarter. |
| 229 | 50 | 2.5 | Streptococcus pyogenes, ..... | R. f. quarter. |
| 230 | 7,250 | 50.+ | Streptococcus pyogenes, ..... | R. f. quarter. |
| 231 | 200 | 50.+ | B. alkaligenes. ..... | L. f. quarter. |
| 232 | 200 | 50.+ | (Culture lost), ..... | R. h. quarter. |
| 233 | 1,000 | ..... | Sarcina lutea, .....<br>Bact. desiduosum, ..... | Infected quarter. |
| 238 | 25 | 50.+ | B. aurescens, ..... | R. f. quarter. |
| 239 | 2,000 | 50.+ | Streptococcus pyogenes, ..... | L. f. quarter. |
| 240 | 0 | 50.+ | ..... | R. h. quarter. |
| 241 | 50 | 50.+ | Staphylococcus albus. | |
| 242 | 0 | 11.7 | | |
| 243 | 25 | 9.0 | B. pseudodlphtherla. | |
| 244 | 100 | 9. | Staphylococcus albus. | |
| 245 | 0 | ..... | ..... | Infected quarter. |
| 246 | 16,300 | ..... | B. pseudodlphtheria, .....<br>Streptococcus pyogenes. | Infected quarter. |
| 247 | 575 | 25.+ | Staphylococcus aureus. | |
| 248 | 1,250 | ..... | Streptococcus pyogenes. | |
| 249 | 53,450 | ..... | Streptococcus pyrogenes, .....<br>B. pseudodiphtherla. | Infected quarter. |
| 250 | 75 | 25.+ | (Culture lost.) | |
| 251 | 25 | 2.3 | (Culture lost.) | |
| 252 | 150 | 0.1 | Bact. exiguum.<br>Staphylococcus aureus. | |
| 253 | 25 | 10 | B. alkaligenes. | |
| 254 | 0 | 22.6 | | |
| 255 | 0 | 100.+ | | |
| 258 | 0 | ..... | | |

TABLE I—Continued.

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 259 | 34,400 | 25.+ | Streptococcus pyogenes. | |
| 260 | 925 | ..... | Streptococcus pyogenes.<br>Staphylococcus aureus. | |
| 261 | 575 | 50.+ | Streptococcus pyogenes.<br>Staphylococcus aureus. | |
| 262 | 0 | 25.+ | | |
| 263 | 675 | 35.+ | (Culture lost.) | |
| 264 | 0 | 25.+ | | |
| 265 | 100 | 35.+ | B. formosum.<br>Staphylococcus aureus. | |
| 266 | 28 | 0.4 | Bact. lactis, ..... | Fresh; r. f. quarter. |
| 267 | 0 | 0.3 | ..... | Fresh; l. f. quarter. |
| 268 | 0 | 25.+ | ..... | Fresh; r. h. quarter. |
| 269 | 0 | 0.2 | ..... | Fresh; l. h. quarter. |
| 270 | 100 | ..... | M. tetragenus.<br>Streptococcus pyogenes. | |
| 271 | 0 | ..... | ..... | Infected quarter. |
| 272 | 400 | ..... | (Culture lost), ..... | Infected quarter. |
| 273 | 1,400 | 25.+ | Streptococcus pyogenes. | |
| 274 | 0 | 0.5 | | |
| 275 | 50 | 0.1 | Staphylococcus albus. | |
| 276 | 50 | 25.+ | ..... | Infected quarter. |
| 277 | 525 | 0.9 | Staphylococcus aureus. | |
| 278 | 25 | 2.0 | ..... | Nearly dry, r. f. |
| 279 | 0 | 50.+ | ..... | Nearly dry, l. f. |
| 280 | 100 | 24.4 | Staphylococcus albus, .....<br>Streptococcus pyogenes, ..... | Nearly dry, r. h. |
| 281 | 50 | 25.+ | Staphylococcus albus, ..... | Nearly dry, l. h. |
| 284 | 2,075 | 25. | M. tetragenus.<br>Streptococcus pyogenes. | |
| 285 | 400 | ..... | Streptothrix.<br>Bact. convolutum. | |
| 291 | 0 | 0.8 | ..... | Milked 2 mo., r. f. |
| 292 | 5,100 | 6.0 | Staphylococcus aureus, ..... | Milked 2 mo., l. f. |
| 293 | 150 | 25.+ | Streptococcus pyogenes, ..... | Milked 2 mo., r. h. |
| 294 | 5,825 | 3.0 | Streptococcus pyogenes, .....<br>B. pseudodiphtheria, ..... | Milked 2 mo., l. h. |
| 295 | 3,400 | 25. | Streptococcus sarcina alba, .....<br>Staphylococcus albus. | Milked 8 mo. r. f. |
| 296 | 7,425 | 4. | Streptococcus pyogenes, ..... | Milked 8 mo., l. f. |
| 297 | 4,200 | 1.2 | Streptococcus pyrogenes, ..... | Milked 8 mo., r. h. |
| 298 | 1,900 | 12 | (Culture lost), ..... | Milked 8 mo., l. h. |
| 299 | 125 | 2.2 | ..... | Milked 3 weeks, r. f. |
| 300 | 8,325 | 25. | Streptococcus pyogenes, .....<br>Staphylococcus albus, ..... | Milked 3 weeks, l. f. |
| 301 | 150 | 22. | B. pseudodiphtheria, .....<br>Staphylococcus albus, ..... | Milked 3 weeks, r. h. |
| 302 | 275 | 7. | B. pseudodiphtheria, ..... | Milked 3 weeks, l. h. |
| 303 | 25 | 1.5 | B. pseudodiphtheria, ..... | Milked 4 mo., r. f. |
| 304 | 25 | 0.8 | Staphylococcus albus, ..... | Milked 4 mo., l. f. |
| 305 | 0 | 0.1 | ..... | Milked 4 mo. r. h. |
| 306 | 75 | 1.2 | Staphylococcus albus, ..... | Milked 4 mo. l. h. |
| 311 | 0 | 1. | | |
| 312 | 100 | 0.1 | Staphylococcus aureus. | |
| 319 | 25,050 | 25.+ | Streptococcus pyogenes.<br>Staphylococcus albus. | |
| 320 | 10,400 | 25.+ | Streptococcus pyogenes. | |
| 321 | 25 | 9. | B. desiduosum, ..... | Fresh. |
| 322 | 150 | 7. | Staphylococcus albus, ..... | |
| 323 | 100 | 11. | Staphylococcus aureus, ..... | |

TABLE I—Continued.

| Number of sample. | Number of bacteria per cc. | Number of leucocytes per field. | Nature of Bacteria Found. | Remarks. |
|---|---|---|---|---|
| 324 | 6,950 | 6. | Staphylococcus albus, | Fresh. |
| 325 | 550 | 50.+ | Staphylococcus albus. | |
| 326 | 175 | 50.+ | Staphylococcus albus. | |
| 327 | 0 | 6. | | |
| 328 | 8,400 | 50.+ | Staphylococcus albus.<br>Streptococcus pyogenes. | |
| 329 | 14,100 | 28. | Bact. convolutum, | Calved evening before. |
| 330 | 8,400 | 9. | B. pseudodiphtheria, | |
| 331 | 3,150 | 50.+ | Bact. fulvum,<br>B. pseudodiphtheria, | |
| 332 | 1,850 | 50.+ | Staphylococcus albus, | |
| 333 | 250 | 4. | Staphylococcus albus, | Calved day before. |
| 334 | 850 | 6. | B. pseudodiphtheria,<br>Staphylococcus albus, | |
| 335 | 175 | 5. | Staphylococcus citreus, | |
| 336 | 44,300 | 9. | Staphylococcus albus, Bact. lacunatum,<br>Streptococcus pyogenes, | |
| 341 | 1,900 | 50.+ | Ps. fluorescens.<br>M. varians lactis.<br>Staphylococcus aureus. | |
| 342 | 100 | 0.2 | B. pseudodiphtheria.<br>Bact. flexuosum. | |

The number of samples of milk of this character that has been examined is 272. The number of bacteria found in these samples ranged from 0 to 93,100 per cubic centimeter. Of the entire number of samples collected directly from the udder, 87, or 32 per cent., contained no bacteria; 118, or 43.64 per cent. of the samples contained less than 500 bacteria; while 28, or 10.29 per cent. of the samples contained over 5,000 bacteria per cubic centimeter.

With regard to the nature of the bacteria found in the samples of milk drawn directly from the udder, several interesting facts were noted. In many samples careful inspection of the plate cultures and isolation and study of all the different species of bacteria noted, revealed the presence of a single species representing almost constantly either one of three organisms, viz: The streptococcus, staphylococcus, or a bacillus of the type of the Bacillus pseudodiphtheria. In other samples, again, two or each of these three organisms were present, though, usually, one of the organisms was present in much larger numbers than the others.

From some of the samples other bacilli were also isolated and studied, but it is probable, from the character of these bacilli, that

they gained access to the milk or to the plate cultures from the orifice of the teat, or from the air of the stable or the laboratory. These bacilli probably represent merely accidental contaminations of the milk or of the plate cultures, because they were encountered usually as single colonies on a plate culture. Moreover, the character of these bacilli is such as to lead one to believe that they would not find suitable conditions for development in the interior of the milk cistern. Several of the bacilli isolated from these samples belong to the class of bacteria usually regarded as air organisms.

The three types of organisms found most frequently in these samples, viz: The streptococcus, staphylococcus, and the Bacillus pseudodiphtheria type, were found in such large numbers as to leave no doubt that they were derived directly from the cow's udder. In many samples one of the three organisms was found in pure culture in enormous numbers. Of the three organisms, the streptococcus was present most frequently, and most of the samples containing over 5,000 bacteria per cubic centimeter contained this organism alone. The interesting relation between the occurrence of large numbers of streptococci and pus cells in some of the samples will be pointed out later.

*2. The nature and the source of the bacteria gaining access to the milk in the ordinary manipulations in collecting and storing milk in modern dairies and milkhouses.*

Special effort was made to isolate from the plate cultures of the samples of milk collected from the various utensils and apparatus all the different species of bacteria that could be detected. Subsequent study of the bacteria isolated from these samples, showed in what respect they differed from those found in samples collected directly from the udder.

The results obtained in the study of these samples of milk are shown in Table II.

TABLE II.

Results obtained with milk collected from various milk utensils:

| Number of sample. | Number of bacteria. | Nature of Bacteria. | Utensils from which collected. Probable source of the bacteria. |
|---|---|---|---|
| 2 | 350 | Staphylococcus aureus, | Bucket. |
| | | B. pseudodiphtheria. | |
| 3 | 23,650 | Micrococcus, | Strainer. |
| | | B. matazoonii, Bact. connii. | |
| | | B. alkaligenes. | |
| 4 | 120,400 | Streptococcus pyogenes, | Cooler. |
| | | B. ellingtonii. | |
| | | B. matazoonii. | |
| | | Bact. connii. | |
| 29 | 25 | Staphylococcus albus, | Udder. |
| 30 | 1,250 | Streptococcus, and staphylococcus citreus, | Bucket. |
| 31 | 21,225 | B. ellingtonii, B. alkeligenes, | Strainer. |
| 32 | 508,200 | Staphylococcus citreus, | Cooler. |
| | | Bact. connii. | |
| | | Bact. desiduosum. | |
| 43 | 12,800 | Staphylococcus albus, | Strainer. |
| | | Streptococcus pyogenes. | |
| | | B. matazoonii. | |
| 44 | 21,700 | Bact. lactis erythrogenes, | Cooler. |
| | | B. pseudodiphtheria. | |
| | | Staphylococcus albus. | |
| 50 | 1,375 | Staphylococcus citreus, | Strainer. |
| | | Sarcina lutea. | |
| 51 | 2,600 | Staphylococcus citreus, | Cooler. |
| | | B. matazoonii. | |
| | | Streptococcus pyogenes. | |
| 52 | 20,075 | Streptococcus pyogenes, | Udder. |
| 53 | 4,200 | Streptococcus pyogenes, | Bucket. |
| | | B. pseudodiphtheria. | |
| 54 | 4,200 | Staphylococcus citreus, | Strainer. |
| | | Streptococcus pyogenes. | |
| 55 | 15,950 | Staphylococcus aureus, | Cooler. |
| | | Streptococcus pyogenes. | |
| 79 | 1,685 | Straphylococcus citreus, | Strainer. |
| 80 | 2,900 | Micrococcus citreus lactis, | Cooler. |
| | | Sarcina alba. | |
| 82 | 8,350 | Staphylococcus albus, | Bucket. |
| | | Streptococcus pyogenes. | |
| 83 | 14,925 | Stapbylo. alb. and streptococcus, | Strainer. |
| 84 | 1,700 | Staphylo. alb. and streptococcus, | Cooler. |
| 196 | 0 | | Udder. |
| 197 | 25 | Staphylococcus albus, | Bucket. |
| 199 | 0 | | Udder. |
| 200 | 75 | Staphylococcus albus, | Bucket. |
| 211 | 4,050 | B. geminus, Bact. middletownii, | Can. |
| | | Bact. connii. | |
| | | B. varians lactis. | |
| 212 | 3,100 | Staphylo, aureus, streptococcus pyogenes, | Tank below strainer. |
| | | Bact. convolutum. | |
| | | Bact. flexuosum. | |
| | | B. alkaligenes. | |
| 213 | 3,900 | Bact. convolutum, B. schafferi, | Cooler. |
| | | Bact. flexuosum, B. communis lactis. | |
| | | B. alkaligenes. | |
| 214 | 9,875 | Staphylococcus albus, | Tank below cooler. |
| | | Bact. desiduosum. | |
| | | Bact. varians lactis. | |

TABLE II—Continued.

| Number of sample. | Number of bacteria. | Nature of Bacteria. | Utensils from which collected. Probable source of the bacteria. |
|---|---|---|---|
| 215 | 1,475 | Bact. middletownii,<br>B. aureus lactis II. | Can. |
| 216 | 1,725 | Staphylococcus aureus, streptococcus pyogenes,<br>B. alkaligenes. | Tank. |
| 217 | 1,625 | Staphylococcus albus streptococcus,<br>Bact. convolutum B. coli.<br>Bact. fairmountensis. | Cooler. |
| 218 | 2,275 | M. tetragenus.<br>Bact. fuloum. | Tank. |
| 234 | 925 | Staphylo. alb. streptococcus pyogenes, | Can. |
| 235 | 425 | Staphylo. alb. streptococcus pyogenes, | Tank. |
| 236 | 400 | Staphylococcus aureus, | Cooler. |
| 237 | 650 | M. tetragenus staphylo. aur., | Tank. |
| 255 | 0 | | Udder. |
| 256 | 325 | Bact. lactis, | Bucket. |
| 257 | 16,200 | Staphylo. aur. streptococcus pyogenes,<br>B. ellingtonii. | Cooler. |
| 285 | 400 | Bact. convolutum, | Udder. |
| 286 | 850 | Staphylococcus aur. strepto. pyogenes, | Bucket. |
| 287 | 60,900 | B. coli. Bact. lactis, | Strainer. |
| 288 | 34,550 | Staphylococcus albus,<br>B. lacticum. B. alkaligenes. | Cooler. |
| 289 | 173,600 | Streptococcus, B. coli,<br>B. acidi lactici. B. geminus.<br>Bact. desiduosum, B. toxigenes. | Tank. |
| 290 | 84,000 | B. ellingtonii, | Bottler. |
| 307 | 3,350 | Staphylo. alb. B. pseudodiph., | Strainer. |
| 308 | 3,250 | Staphylo. alb. streptococcus, | Cooler. |
| 309 | 5,850 | Strepto. M. tetragenus, | Tank. |
| 310 | 4,750 | Staphylococcus aureus,<br>Bact. acidi lactici.<br>Bact. convolutum. | Bottler. |
| 312 | 100 | Staphylococcus aureus, | Uddder. |
| 313 | 4,850 | Staphylococcus albus,<br>B. acidi lactici. | Bucket. |
| 314 | 24,200 | Diplococcus, | Strainer. |
| 315 | 3,200 | Staphylococcus aureus, | Tank. |
| 316 | 4,550 | Streptococcus pyogenes, | Cooler. |
| 317 | 1,950 | Staphylococcus aureus, | Tank. |
| 337 | 1,900 | Staphylococcus albus, | Strainer. |
| 338 | 1,350 | Diplococcus, | Tank. |
| 339 | 1,400 | Streptococcus pyogenes,<br>Bact. acidi lactici. | Cooler. |
| 340 | 2,550 | Staphylococcus albus,<br>Streptococcus pyogenes. | Tank. |

The samples of milk collected from the various utensils and apparatus, contained primarily the bacteria derived from the cow's udder, and, in addition to these, a number of other species were found which gained access during the milking, straining, cooling, and bottling of the milk. It is, therefore, not necessary to discuss further the bacteria primarily contained in the milk, as these have already been considered in the first section of this report.

Reference to Table II will show that the bacteria gaining access to the milk during the ordinary manipulations in modern dairies and milkhouses, are derived, in very large part, from the milking utensils and apparatus through which the milk passes in the straining and cooling process. Analyses of samples collected from the first milk cooled, show that this milk usually contains much greater numbers of bacteria than samples collected after 50 to 100 quarts of milk have been cooled. These facts seem to point to the straining and cooling apparatus as the most fruitful source of bacteria in the milk. In those dairies in which the straining, cooling and bottling apparatus was sterilized with steam just prior to cooling the milk, the character of the bacteria found in the samples did not differ from those found in samples derived directly from the cow's udder. It is probable, therefore, that the organisms which gain access to milk in cooling, are either air bacteria, or organisms derived from the water used in washing the apparatus, or such organisms as may be deposited on the apparatus by flies.

Samples Nos. 31 and 32 were collected from the strainer and cooler, respectively, and represent the first milk passing over. Samples Nos. 43 and 44 were collected from the same source after about 50 quarts of milk had passed over, while samples Nos. 50 and 51 were collected after about 100 quarts of milk had passed over.

Samples Nos. 54 and 55 were collected from the strainer and cooler, respectively, in another dairy, and represent the first milk passing over. Samples Nos. 79 and 80 were collected from the same source after about 50 quarts of milk had passed over.

Samples Nos. 83 and 84 were collected from the strainer and cooler, respectively, in a third dairy, and represent the first milk passing over. Samples Nos. 102 and 103 were collected from the same source after about 60 quarts of milk had passed over.

Sample No. 257 was collected from the cooler in a fourth dairy and represents the first milk passing over. Sample No. 282 was collected from the same source after about 100 quarts of milk had passed over .

Samples Nos. 287 to 290 were collected from the strainer, cooler, tank below cooler, and bottler, respectively, in a fifth dairy, and represent the first milk passing over. Samples Nos. 307 to 310 were collected from the same source after about 200 quarts of milk had passed over.

Samples Nos. 314 to 317 were collected from the strainer, tank below strainer, cooler, and tank below cooler, respectively, in a sixth dairy, and represent the first milk passing over. Samples Nos. 337 to 340 were collected from the same source after about 100 quarts of milk had passed over.

The difference in the number of bacteria, obtained in samples

collected from the straining, cooling and bottling apparatus at the beginning of the milking and those collected after from 50 to 100 quarts of milk had passed over the apparatus, is shown in Table II (a). In most instances a marked increase in the number of bacteria is noted as the milk passes through the apparatus. Those instances, in which no such marked increase in the number of bacteria was encountered, represent dairies in which the buckets, cans, straining, cooling and bottling apparatus were sterilized immediately before beginning the milking. Where all the utensils and apparatus were sterilized with steam before beginning the milking, the bacteria contained in samples collected from the various utensils and apparatus differed very little, comparatively speaking, from the samples collected directly from the cows.

The nature of the bacteria found in these samples of milk is shown in Table II. The organisms are of several different species. In some of the samples Bacillus coli was found, but the occurrence of this organism was rather infrequent. By far the larger number of the organisms found in these samples belong to the class of putrefactive bacteria as shown by the alkaline reaction produced in the milk cultures and by the liquefaction of gelatin. Several of the organisms found are water bacteria and probably gained access to the milk utensils through the water used in washing. The absence of members of the Bacillus lactis group from most of the samples seems somewhat unusual, as these bacteria were encountered in only a few of the samples.

The bacteria isolated from these samples of milk do not belong in the class of disease-producing organisms, but seem to belong more particularly to the group of organisms which cause the milk to putrefy. They may be highly injurious, however, because they may have the property of forming poisonous metabolic products (ptomaines) when growing in milk.

TABLE II (a).

Shows the results of the analysis of samples of milk collected from the straining, cooling and bottling apparatus at the beginning of the milking, and again after some milk had passed over the same:

| | Bucket. | Strainer. | Cooler. | Tank below cooler. | Bottler. |
|---|---|---|---|---|---|
| Beginning, | 350 | 23,650 | 120,400 | ..... | ..... |
| Beginning, | 1,250 | 21,225 | 508,200 | ..... | ..... |
| After 100 quarts, | ..... | 1,375 | 2,600 | ..... | ..... |
| Beginning, | 4,200 | 4,200 | 15,950 | ..... | ..... |
| After 50 quarts, | ..... | 1,685 | 2,900 | ..... | ..... |
| Beginning, | 8,350 | 14,925 | 1,700 | ..... | ..... |
| After 60 quarts, | ..... | 12,325 | 8,800 | ..... | ..... |
| | Can. | | | | |
| Beginning, | 4,050 | 3,100 | 3,900 | 9,875 | ..... |
| After 60 quarts, | 1,475 | 1,725 | 1,625 | 2,275 | ..... |
| Beginning, | 925 | 425 | 400 | 650 | ..... |
| | Bucket. | | | | |
| Beginning, | 850 | 60,900 | 34,550 | 173,600 | 84,000 |
| After 200 quarts, | ..... | 3,350 | 3,250 | 5,850 | 4,750 |
| Beginning, | 4,850 | 24,200 | 4,550 | 1,950 | ..... |
| After 100 quarts, | ..... | 1,900 | 1,400 | 2,550 | ..... |

TABLE III.

Showing the prevalence of streptococci in milk, and the relation of these bacteria to the occurrence of pus in milk, and to the general bacterial content of milk drawn directly from the udder:

| Number of sample. | Number of bacteria found. | Number of pus cells found. | Occurrence of streptococci in pure culture. | Other Species of Bacteria Also Present. |
|---|---|---|---|---|
| 9 | 11,425 | 40. | Pure culture. | |
| 17 | 1,300 | 1. | Pure culture. | |
| 49 | 300 | 0.02 | ..... | Bact. convolutum, Bact. connii, Staphylococcus citreus. |
| 52 | 20,075 | 0.2 | Pure culture. | |
| 67 | 8,800 | 1.0 | Pure culture. | |
| 89 | 25 | 0.6 | Pure culture. | |
| 91 | 150 | 1.3 | ..... | Staphylococcus aureus and albus. |
| 97 | 10,475 | 50. | Pure culture. | |
| 106 | 1,050 | 14.3 | Pure culture. | |
| 109 | 775 | 10.2 | Pure culture. | |
| 114 | 475 | 35. | ..... | B. pseudodiphtheria. |
| 115 | 100 | 12.4 | ..... | Staphylococcus albus. |

TABLE III—Continued.

| Number of sample. | Number of bacteria found. | Number of pus cells found. | Occurrence of Streptococci in pure culture. | Other Species of Bacteria Also Present. |
|---|---|---|---|---|
| 119 | 93,100 | 8.6 | Pure culture. | |
| 133 | 200 | 0.4 | Pure culture. | |
| 135 | 5,100 | 3.8 | Pure culture. | |
| 142 | 13,950 | 8.0 | Pure culture. | |
| 143 | 6,425 | 50.+ | ..... | M. tetragenus. |
| 145 | 1,700 | 25. | Pure culture. | |
| 148 | 775 | 12. | ..... | B. alkaligenes. |
| 153 | 3,550 | 50.+ | ..... | B. pseudodiphtheria. |
| 175 | 34,725 | 50.+ | Pure culture. | |
| 180 | 2,075 | 50.+ | ..... | Bact. circulans. |
| 183 | 5,225 | 26.3 | Pure culture. | |
| 191 | 4,950 | 15. | Pure culture. | |
| 209 | 2,200 | 35.+ | Pure culture. | |
| 219 | 67,050 | ..... | Pure culture. | |
| 229 | 50 | 2.5 | Pure culture. | |
| 230 | 7,250 | 50.+ | Pure culture. | |
| 239 | 2,000 | 50.+ | Pure culture. | |
| 246 | 16,300 | ..... | ..... | B. pseudodiphtheria. |
| 248 | 1,250 | ..... | Pure culture. | |
| 249 | 53,450 | ..... | ..... | B. pseudodiphtheria. |
| 259 | 34,400 | 25.+ | Pure culture. | |
| 260 | 925 | ..... | ..... | Staphylococcus aureus. |
| 261 | 575 | 50.+ | ..... | Staphylococcus aureus. |
| 270 | 100 | ..... | ..... | M. tetragenus. |
| 273 | 1,400 | 25.+ | Pure culture. | |
| 280 | 100 | 24.4 | ..... | Staphylococcus albus. |
| 284 | 2,075 | 25. | ..... | M. tetragenus. |
| 285 | 400 | ..... | ..... | Bact. convolutum. |
| 293 | 150 | 25.+ | Pure culture. | |
| 294 | 5,825 | 3.0 | ..... | B. pseudodiphtheria. |
| 295 | 3,400 | 25. | ..... | Staphylococcus albus, sarcina alba. |
| 296 | 7,425 | 4. | Pure culture. | |
| 297 | 4,200 | 1.2 | Pure culture. | |
| 300 | 8,325 | 25. | ..... | Staphylococcus albus. |
| 319 | 25,050 | 25.+ | ..... | Staphylococcus albus. |
| 320 | 10,400 | 25.+ | Pure culture. | |
| 328 | 8,400 | 50.+ | ..... | Staphylococcus albus. |
| 336 | 44,300 | 9. | ..... | Staphylococcus albus. |

*3. The occurrence of leucocytes and pus cells in the milk of healthy cows, and a determination of the significance of their presence in the milk.*

The estimation of the number of leucocytes and pus cells in the milk was made by centrifuging 10 cubic centimeters of the milk and spreading the sediment in a thin film on a clean coverslip. These coverslip preparations were dried, fixed by passing rapidly through the flame, and then treated with chloroform. The chloroform was poured off and after the films had again dried they were stained with Loeffler's alkaline methylene blue solution and mounted

on slides. The number of cells in a film was determined by counting ten fields with the 1-12 immersion lens and dividing the result obtained by 10. The number of cells found in samples collected from individual cows may be seen by reference to Tables I and III. In the latter table the relation of the occurrence of pus in milk to the streptococcus content is shown in parallel columns.

There is still no agreement among bacteriologists as to the number of cells in a specimen that will justify the diagnosis of the presence of pus. The number of cells in a field of the 1-12 immersion lens is taken arbitrarily at ten. This number of cells per field may not always indicate pus, but it is believed that in the majority of instances, it does indicate the presence of pus in milk derived from individual cows.

Reference to Table III will show that the majority of the samples containing large numbers of streptococci also contained pus cells, that is, more than ten cells per field. The fact that some of the samples contained large numbers of streptococci with less than ten cells per field, indicates that there was some pus in these samples also, and that, therefore, a standard must be selected somewhat arbitrarily. It is believed, on these grounds, that ten cells per field is a safe limit for milk collected from a single cow.

Practically, all samples of milk were found to contain some cellular elements, and hence the number of cells along with the presence of pyogenic organisms must be taken as the most important criterion of the character of the milk. It is impossible, by any of the methods known at present, to differentiate positively between a leucocyte and a pus cell, as the latter is merely a dead leucocyte. The number of cells must, therefore, be taken into consideration. If pus is present in milk, the cells are also usually present in small masses, while the leucocytes always occur as isolated cells.

TABLE IV.

Results obtained with samples of milk collected separately from each of the four quarters of the udder:

| Number of sample. | Quarter of the udder. | Number of bacteria. | Number of pus cells. | Bacteria Seen on Microscopic Examination of Sediment. |
|---|---|---|---|---|
| 226 | Right hind, | 0 | 3.1 | |
| 227 | Left hind, | 100 | 6.1 | |
| 228 | Left front, | 50 | 0.9 | |
| 229 | Right front, | 50 | 2.5 | |
| 230 | Right front, | 7,250 | 50.+ | |
| 231 | Left front, | 200 | 50.+ | |
| 232 | Right hind, | 200 | 50.+ | |
| 233 | Left hind, | 1,000 | ..... | |
| 238 | Right front, | 25 | 50.+ | |
| 239 | Left front, | 2,000 | 50.+ | |
| 240 | Right hind, | 0 | 50.+ | |
| 241 | Left hind, | 50 | 50.+ | |
| 242 | Right front, | 0 | 11.7 | |
| 243 | Left front, | 25 | 9.0 | |
| 244 | Right hind, | 100 | 9.0 | |
| 245 | Left hind, | 0 | ..... | |
| 246 | Right front, | 16,300 | ..... | |
| 247 | Left front, | 575 | 25.+ | |
| 248 | Right hind, | 1,250 | ..... | |
| 249 | Left hind, | 53,450 | ..... | Streptococcus pyogenes. |
| 250 | Right front, | 75 | 25.+ | |
| 251 | Left front, | 25 | 2.3 | |
| 252 | Right hind, | 150 | 0.1 | |
| 253 | Left hind, | 25 | 10.0 | |
| 258 | Right front, | 0 | ..... | Streptococcus pyogenes. |
| 259 | Left front, | 34,400 | 25.+ | Streptococcus pyogenes. |
| 260 | Right hind, | 925 | ..... | Streptococcus pyogenes. |
| 261 | Left hind, | 575 | 50.+ | Streptococcus pyogenes. |
| 262 | Right front, | 0 | 25.+ | |
| 263 | Left front, | 675 | 35.+ | |
| 264 | Right hind, | 0 | 25.+ | |
| 265 | Left hind, | 100 | 35.+ | |
| 266 | Right front, | 75 | 0.4 | |
| 267 | Left front, | 0 | 0.3 | |
| 268 | Right hind, | 0 | 25.+ | |
| 269 | Left hind, | 0 | 0.2 | |
| 270 | Right front, | 100 | ..... | Streptococcus pyogenes. |
| 271 | Left front, | 0 | ..... | |
| 272 | Right hind, | 400 | ..... | Streptococcus pyogenes. |
| 273 | Left hind, | 1,400 | 25.+ | Streptococcus pyogenes. |
| 274 | Right front, | 0 | 0.5 | |
| 275 | Left front, | 50 | 0.1 | |
| 276 | Right hind, | 50 | 25.+ | |
| 277 | Left hind, | 525 | 0.9 | |
| 278 | Right front, | 25 | 2.0 | |
| 279 | Left front, | 0 | 50.+ | |
| 280 | Right hind, | 3,825 | 24.4 | |
| 281 | Left hind, | 3,500 | 25.+ | |
| 291 | Right front, | 0 | 0.8 | |
| 292 | Left front, | 5,100 | 6.0 | |
| 293 | Right hind, | 150 | 25.+ | |
| 294 | Left hind, | 5,825 | 3.0 | |
| 295 | Right front, | 3,400 | 25.0 | |
| 296 | Left front, | 7,425 | 4.0 | |
| 297 | Right hind, | 4,200 | 1.2 | |

TABLE IV—Continued.

| Number of sample. | Quarter of the udder. | Number of bacteria. | Number of pus cells. | Bacteria Seen on Microscopic Examination of Sediment. |
|---|---|---|---|---|
| 298 | Left hind, | 1,900 | 12.0 | |
| 299 | Right front, | 125 | 2.2 | |
| 300 | Left front, | 8,325 | 25.0 | |
| 301 | Right hind, | 150 | 22.0 | |
| 302 | Left hind, | 275 | 7.0 | |
| 303 | Right front, | 25 | 1.5 | |
| 304 | Left front, | 25 | 0.8 | Streptococcus pyogenes. |
| 305 | Right hind, | 0 | 0.1 | |
| 306 | Left hind, | 75 | 1.2 | |
| 321 | Right front, | 25 | 9.0 | |
| 322 | Left hind, | 150 | 7.0 | |
| 323 | Right hind, | 0 | 11.0 | |
| 324 | Left hind, | 6,950 | 6.0 | |
| 325 | Right front, | 550 | 50.+ | |
| 326 | Left front, | 175 | 50.+ | |
| 327 | Right hind, | 0 | 6.0 | |
| 328 | Left hind, | 8,400 | 50.+ | |
| 329 | Right front, | 14,100 | 28.0 | |
| 330 | Left front, | 8,400 | 9.0 | |
| 331 | Right hind, | 3,150 | 50.+ | |
| 332 | Left hind, | 1,850 | 50.+ | |
| 333 | Right front, | 250 | 4.0 | |
| 334 | Left front, | 850 | 6.0 | |
| 335 | Right hind, | 175 | 5.0 | Bact. aocunatum. |
| 336 | Left hind, | 44,300 | 9.0 | Bact. lactis. |

*4. The prevalence of streptococci in milk and a determination of the relation of these bacteria to inflammatory conditions of the udder.*

The prevalence of streptococci in milk may be seen by reference to Table III, in which are tabulated the results obtained with the samples of milk in which streptococci were found.

The presence of considerable numbers of streptococci in milk is probably always associated with inflammatory conditions of one or more quarters of the udder, while the milk coming from the other quarters may be free from both streptococci and pus cells. This relation of streptococci and pus cells to particular quarters of the udder is shown in Table IV, in which are tabulated the results obtained with samples collected separately from each of the four quarters.

In many instances in which large numbers of streptococci and pus cells were encountered in the milk of a cow it was found that there was present in one or more quarters of the udder some evidence of inflammatory reaction, such as swelling or hardness of

the quarter. Frequently a cow presenting such a condition had suffered sometime previously from an attack of mammitis. In other instances no alteration of the udder could be detected and there was no history of an attack of mammitis elicited. It is probable, therefore, that the inflammatory reaction in the udder may have been so slight as to escape notice, or of such long standing as to have been forgotten by the attendants.

The Swiss, French and Germans distinguish two forms of mammitis, (a), the ordinary catarrhal form which is sporadic in character, and, (b), the contagious mammitis, or "Gelbegalt" of the Swiss, which is readily conveyed from one cow to another and leads to destruction of the function of the udder. Streptococci are usually the cause of both forms of mammitis, though the catarrhal form may also be caused by the staphylococcus. The contagious form of the disease is always due to the streptococcus. The Swiss and French bacteriologists have described a particular form of streptococcus as the cause of the contagious mammitis. This streptococcus has the property of growing out in long chains and ferments lactose with the evolution of carbon dioxid.

In the cases of catarrhal mammitis which were encountered, streptococci, staphylococci and the Bacillus pseudodiphtheria were found, though the streptococcus was found most frequently either alone or in association with either the staphylococcus or the Bacillus pseudodiphtheria, or both.

In the milk of three cows suffering from advanced contagious mammitis, a form of streptococcus was encountered which differed from the streptococcus in the catarrhal form of the disease in the fact that it possessed the property of producing a yellow pigment when grown in agar or milk in the absence of oxygen. This pigment-producing property of the streptococcus of contagious mammitis no doubt gives rise to the peculiar yellow color of the milk in this disease. The cultures of streptococcus isolated from cases of contagious mammitis at no time manifested the property of fermenting lactose which has been noted by the Swiss and French observers.

Since the presence of considerable numbers of pus cells and streptococci in cow's milk is generally believed to be detrimental to health, it is of the greatest importance to have the milk of all cows in modern dairies examined for these constituents. On account of the great tendency of the streptococci to persist for a long time after the subsidence of the symptoms of active catarrhal inflammation, it would be preferable to exclude all cows from the dairy which yield milk rich in pus cells and streptococci. If this were done it would no doubt serve to reduce the frequency of the occurrence of catarrhal mammitis in dairy herds. Along with this

measure it will be of the greatest importance to look carefully after the health of the attendants and milkers so as to prevent the infection of the cows with bacteria which these attendants may carry on their hands. These preventive measures should include the exclusion from the stables of all attendants suffering from sore hands or sore throat, as many of the sore throats in human beings are caused by streptococci. From the fact that streptococci occur in the throats of practically all healthy persons, the thorough cleansing of the hands of the attendants before milking should be rigidly enforced.

The occurrence of contagious mammitis leaves but one safe course of procedure, that is, the prompt removal of the infected cows from the herd. Such cows should never be tended by the regular attendants in the dairy on account of the danger of conveying the disease to healthy cows. Because of the great destruction of the glandular function of the udder as the result of this disease, there is but slight probability that a cow once infected with the disease will ever become profitable again. So far no positive cure has been discovered for this condition.

The practice prevailing in some dairies, of allowing the fore-milk to flow on the floor of the stable, should be prohibited, because of the danger of disseminating infectious bacteria by this means. It is well-known that the fore-milk is more likely to contain large numbers of bacteria than that higher up in the milk cistern. Consequently the fore-milk from each cow should be carefully collected in a special utensil and subsequently boiled to destroy the bacteria contained in it. Likewise all the milk from infected quarters of the udder should be collected and treated in a similar manner. Such milk may then be employed with safety for feeding pigs or young cattle.

In several of the samples of milk collected from the different quarters of the udder (see Table IV), it was found on microscopic examination of the sediment that pus cells were present in countless numbers, though the cultures showed no bacteria. In several of the samples in question streptococci were seen in the coverslips prepared from the sediment. There is no doubt that in all the samples in which such large numbers of pus cells were found streptococci were also present, and would have been demonstrated on repeating the examination.

In several of the samples containing a large number of pus cells, neither streptococci nor staphylococci, the ordinary pus-producing bacteria, were found in the cultures. On the other hand, these samples contained pure cultures of an organism which has some of the characters of the Bacillus diphtheriae, and hence is called the Bacillus pseudodiphtheria. The exact significance of the occurrence

of these bacilli in milk can not be stated at present. They are similar to a large group of related organisms which are somewhat widely distributed in nature and occur quite frequently on the skin and mucous membranes of man and some of the domestic animals, especially in cows. These bacilli are quite common in vaccine virus, and, from the fact that they are frequently encountered in milk, (see Eyre, Brit. Med. Congress, 1901), it is evident that they occur quite commonly on the skin and mucous membranes of cows. Organisms belonging in this group have been found repeatedly in suppurations in human beings, and, recently, I encountered them in abscesses in white mice. Hence it is believed that they are pyogenic in character and may also be the cause of a form of mammitis in cattle. It is probable that they are not the primary cause of the inflammation, but, that when the inflammation has once started, they assist in keeping up the condition. This opinion is based on the fact that these bacilli were more frequently found in association with streptococci or staphylococci, or both, than in the absence of these organisms.

*Relation of period of lactation to the bacterial content of milk and to the prevalence of pus cells in such milk.*

A number of cows were examined at different periods of lactation in order to determine whether any influence upon the bacterial content of the milk could be noted, or upon the presence of pus cells in such milk. The results obtained are shown in Tables V and VI.

TABLE V.

Shows the results of the analyses of samples of milk derived from cows at different periods of lactation:

| Number of sample. | Period of lactation. | Number of bacteria. | Number of leucocytes and pus cells. | Nature of Bacteria Found. |
|---|---|---|---|---|
| 329 | Calved evening before, ..... | 14,100 | 28.0 | Bact. convolutum. |
| 330 | Calved evening before, ..... | 8,400 | ..... | Bact. convolutum. |
| 331 | Calved evening before, ..... | 3,150 | 50.+ | B. pseudodiphtheria.<br>B. Bact. convolutum. |
| 332 | Calved evening before, ..... | 1,850 | 50.+ | Bact. flexuosum.<br>B. pseudodiphtheria. |
| 333 | Calved the day before, ..... | 250 | 4.0 | Staphylococcus albus.<br>Staphylococcus albus. |
| 334 | Calved the day before, ..... | 850 | 6.0 | B. pseudodiphtheria. |
| 335 | Calved the day before,..... | 175 | 5.0 | Staphylococcus albus and aureus.<br>Staphylococcus citreus. |

TABLE V—Continued.

| Number of sample. | Period of lactation. | Number of bacteria. | Number of leucocytes and pus cells. | Nature of Bacteria Found. |
|---|---|---|---|---|
| 336 | Calved the day before,..... | 44,300 | 9.0 | Streptococcus pyogenes.<br>Bact. lacunatum.<br>Bact. lactis. |
| 136 | Calf removed same day,..... | 0 | 25.0 | |
| 157 | Calf removed 5 days, ..... | 350 | 0.6 | B. pseudodiphtheria.<br>Bact. deciduosum. |
| 156 | Calf removed 8 days, ..... | 0 | 9.5 | |
| 86 | Nearly fresh, ..... | 0 | 8.1 | |
| 62 | Nearly fresh, ..... | 0 | 0. | |
| 46 | Fresh, ..... | 800 | 1.0 | (Culture lost.) |
| 111 | Nearly fresh, ..... | 25 | 0.1 | Staphylococcus albus. |
| 266 | Fresh, ..... | 75 | 0.4 | Bact. lactis. |
| 267 | Fresh, ..... | 0 | 0.3 | |
| 268 | Fresh, ..... | 0 | 25.+ | |
| 269 | Fresh, ..... | 0 | 0.2 | |
| 341 | 3 weeks after calving, ..... | 1,900 | 50.+ | B. pseudodiphtheria.<br>Bact. varians lactis.<br>Staphylococcus aureus. |
| 299 | Milked three weeks, ..... | 125 | 2.2 | Micrococcus. |
| 300 | Milked three weeks, ..... | 8,325 | 25.0 | Streptococcus pyogenes. |
| 301 | Milked three weeks, ..... | 150 | 22.0 | Staphylococcus albus. |
| 302 | Milked three weeks, ..... | 275 | 7.0 | Staphylococcus albus.<br>B. pseudodiphtheria. |
| 291 | Milked two months, ..... | 0 | 0.8 | B. pseudodiphtheria. |
| 292 | Milked two months, ..... | 5,100 | 6.0 | Staphylococcus aureus. |
| 293 | Milked two months, ..... | 150 | 25.+ | Streptococcus pyogenes. |
| 294 | Milked two months, ..... | 5,825 | 3.0 | Streptococcus pyogenes.<br>B. pseudodiphtheria. |
| 303 | Milked four months,..... | 25 | 1.5 | B. pseudodiphtheria. |
| 304 | Milked four months,..... | 25 | 0.8 | Staphylococcus albus. |
| 305 | Milked four months,..... | 0 | 0.1 | |
| 306 | Milked four months,..... | 75 | 1.2 | Staphylococcus albus. |
| 295 | Milked eight months,..... | 3,400 | 25.0 | Staphylococcus albus.<br>Sarcina alba streptococcus pyogenes. |
| 296 | Milked eight months,..... | 7,425 | 4.0 | Streptococcus pyogenes. |
| 297 | Milked eight months,..... | 4,200 | 1.2 | Streptococcus pyogenes. |
| 298 | Milked eight months,..... | 1,900 | 12.0 | (Culture lost.) |
| 278 | Nearly dry, ..... | 25 | 2.0 | Streptococcus pyogenes, staphylococcus albus. |
| 279 | Nearly dry, ..... | 0 | 50.+ | Staphylococcus albus. |
| 280 | Nearly dry, ..... | 3,825 | 24.4 | B. pseudodiphtheria. |
| 281 | Nearly dry, ..... | 3,500 | 25.+ | Bact. coli. |
| 110 | Nearly dry, ..... | 150 | 3.1 | (Culture lost.) |
| 88 | Nearly dry, ..... | 875 | 19.0 | |
| 87 | Nearly dry, ..... | 100 | 0.2 | |
| 61 | Nearly dry, ..... | 0 | 4.1 | |
| 58 | Nearly dry, ..... | 3,250 | 1.5 | Staphylococcus albus. |
| 205 | Within 2 months of term,.. | 575 | 25.+ | Staphylococcus albus. |
| 35 | Within 6 weeks of term,.... | 850 | 1.0 | M. tetragenus.<br>Staphylococcus albus. |
| 159 | Within 5 weeks of term,.... | 850 | 18.0 | B. pseudodiphtheria.<br>Staphylococcus albus. |

TABLE VI.

Shows the results of the analysis of the milk drawn from the udder of a single cow before and after calving:

COW NO. 5.

| Date. | Period of lactation. | Number of bacteria. | Number of leucocytes and pus cells. | Nature of Bacteria Found. |
|---|---|---|---|---|
| 1903. | | | | |
| July 20 | 7 weeks before calving,.... | 575 | 25.+ | Staphylococcus. |
| Sept. 14 | Calved the evening before,. | 14,100 | 28.0 | Bact. convolutum. |
| Sept. 14 | Calved the evening before,. | 8,400 | 9.0 | B. pseudodiphtheria. |
| Sept. 14 | Calved the evening before,. | 3,150 | 50.+ | Bact. fulvum. |
| Sept. 14 | Calved the evening before,. | 1,850 | 50.+ | B. pseudodiphtheria.<br>Staphylococcus. |
| Oct. 7 | 3 weeks after calving,..... | 1,900 | 50.+ | Ps. fluorescens.<br>B. pseudodiphtheria.<br>Staphylococcus. |
| Nov. 19 | 9 weeks after calving,..... | 2,450 | 10.0 | Staphylococcus albus.<br>Staphylococcus aureus. |
| Nov. 20 | 9 weeks after calving,..... | 4,825 | 25.+ | Staphylococcus albus.<br>B. acidi lactici. |
| 1904. | | | | |
| Jan. 7 | 4 months after calving,.... | 1,350 | 4.2 | Staphylococcus albus.<br>Staphylococcus aureus |
| Jan. 8 | 4 months after calving,..... | 3,150 | 25.+ | Staphylococcus albus.<br>Staphylococcus aureus. |

COW NO. 82.

| Date. | Period of lactation. | Number of bacteria. | Number of leucocytes and pus cells. | Nature of Bacteria Found. |
|---|---|---|---|---|
| 1903. | | | | |
| Sept. 14 | Calved the day previous,... | 250 | 4.0 | Staphylococcus albus. |
| Sept. 14 | Calved the day previous,... | 850 | 6.0 | Staphylococcus albus. |
| Sept. 14 | Calved the day previous,... | 175 | 5.0 | B. pseudodiphtheria.<br>Staphylococcus albus. |
| Sept. 14 | Calved the day previous,... | 44,300 | 9.0 | Staphylococcus citreus. |
| Oct. 7 | 3 weeks after calving,..... | 100 | 0.2 | Bact. flexuosum.<br>B. pseudodiphtheria. |
| Nov. 21 | 9 weeks after calving,..... | 2,225 | 0.4 | Staphylococcus citreus.<br>B. citreus lactis. |
| Nov. 22 | 9 weeks after calving,..... | 4,725 | 0.3 | M. varians lactis.<br>B. ruber lactis. |

From the results obtained, it is evident that the period of lactation has no direct influence upon the occurrence of pus cells and streptococci in the milk. Samples were collected from cows just before, and just after calving, and at various periods of the lactation without discovering any constancy in the results. In several instances where samples of milk were collected from a cow some time before calving, and again at intervals of about a month subsequent to calving, it was found that if the milk was rich in pus cells and bacteria before calving, it showed the same characters after calving, except that there was a gradual diminution of both pus cells and bacteria as time went on (see Table VI).

It seems evident that other factors than the period of lactation influence the occurrence of pus cells and streptococci in a cow's milk. These factors are most probably direct infection from another cow through the hands of the attendants. If this is the case, then it is probable that a cow would be most readily infected at such a time as when her health is most easily disturbed, that is, at the time of calving. Experience has shown, however, that this is not the only time at which cows are liable to contract inflammation of the udder. Such infection may occur at anytime during the period of lactation when the cow's health becomes disturbed through over-feeding, exposure to cold and dampness, and numerous other conditions.

Only one cow was found, in the different dairies visited, which was in heat. The examination of the milk of this cow revealed nothing that was abnormal (see Sample No. 23, Table I).

*5. The relation of the streptococci found in milk to those encountered in scarlet fever, diphtheria, rheumatism, erysipelas and other diseases of man and the domestic animals.*

Several of the cultures of streptococcus isolated from different samples of milk which appeared to differ slightly from each other in their morphologic and biologic characters, as well as a culture of streptococcus isolated from the case of scarlet fever, were used in the immunization of different goats in order to determine whether the cultures could be definitely differentiated from each other by means of the agglutination test.

The agglutination tests were made in test tubes, as experience had shown that this was a more reliable and satisfactory method for demonstrating the agglutination reaction with streptococci than the ordinary hanging-drop method. The results obtained in these tests are shown in Table VII.

TABLE VII.

Shows the results obtained in the agglutination tests with the sera of goats immunized against different cultures of streptococcus:

| Culture of Streptococcus. | Immune Sera. | | | | |
|---|---|---|---|---|---|
| | 17.1† | 43.5 | S-F | 219.1 | 84.3 |
| Milk 3.6,† | | | | 10 | 10 |
| Milk 5.2, | | 10 | 5 | | |
| Milk 9.1, | 50 | | 10 | 50 | 50 |
| Milk 17.1, | 100 | 20 | | 100 | 20 |
| Milk 18.2, | | | 20 | | |
| Milk 43.3, | 10 | | 5 | | |
| Milk 43.5, | *10 | 10 | 5 | 10 | 10 |
| Milk 52.2, | | | | 50 | 100 |
| Milk 53.1, | | | | 100 | 100 |
| Milk 54.1, | *10 | | | | |
| Milk 67.2, | | | | 100 | 100 |
| Milk 83.2, | | | | 10 | |
| Milk 84.2, | *10 | | 5 | 10 | 100 |
| Milk 102.2, | *10 | | | | |
| Milk 102.3, | | | | 50 | 100 |
| Milk 103.2, | | | | 10 | 10 |
| Milk 106.1, | | | | 20 | 50 |
| Milk 135.1, | | | | 100 | |
| Milk 180.1, | 50 | | | | |
| Milk 183.2, | *10 | | | | |
| Milk 194.2, | 100 | | | | |
| Milk 197.1, | 20 | | | | |
| Milk 209.1, | 100 | | 10 | | |
| Milk 212.6, | 50 | | | | |
| Milk 216.5, | | | | 100 | |
| Milk 217.6, | 50 | | 20 | | |
| Milk 219.1, | 50 | 5 | 10 | 100 | 20 |
| Milk 230.1, | 50 | | | | |
| Milk 234.2, | 50 | | | | |
| Milk 234.3, | | | | 100 | |
| Milk 235.2, | 50 | | | | |
| Milk 235.3, | 100 | 10 | 20 | | |
| Milk 235.4, | *10 | | | | |
| Milk 237.1, | 50 | | 10 | | |
| Milk 239.1, | 100 | | | | |
| Milk 239.2, | 100 | | | | |
| Milk 246.1, | 100 | | 20 | | |
| Milk 248.1, | 10 | | | | 10 |
| Milk 248.2, | | | | 100 | |
| Milk 249.1, | | | | 50 | 50 |
| Milk 249.2, | 100 | | 10 | | |
| Milk 259.1, | | | | 50 | 50 |
| Milk 260.3, | 20 | | | | |
| Milk 261.1, | | | | 20 | |
| Milk 273.1, | | | | 50 | |
| Milk 286.2, | | | | 50 | 50 |
| Milk 316.1, | | | | 20 | 10 |
| Milk 319.1, | 100 | 10 | | | |
| Milk 319.4, | 50 | | | | |
| Milk 320.1, | | | | 20 | 20 |
| Milk 320.2, | 100 | | | | |
| Milk 328.2, | 100 | 5 | | | |
| Milk 339.1, | | | | 100 | 50 |
| Milk 340.1, | 100 | | | | |
| Scarlet fever, | *10 | | 200 | | 20 |
| Bursa, | | | | 100 | 100 |
| Finger abscess, | | | | 100 | 100 |
| Normal lung, | | | | 100 | 100 |
| Tubercular sputum—3, | | | | 50 | 100 |
| Tubercular sputum—5, | | | | 50 | 20 |

*Signifies that the agglutination reaction was incomplete at the figure indicated. The figures represent the extent to which the serum of the animal was diluted, e. g., 100 indicates that one part of serum was diluted with 99 parts of salt solution.

†The whole number represents the number of the sample of milk from which the culture was derived, while the decimal represents the first, second, etc., culture isolated from such sample, e. g., 84.3 represents culture No. 3 isolated from sample No. 84.

While some differences in the degree of agglutination are to be observed with the different cultures, yet these differences are merely relative and it is believed that they do not indicate that the streptococci are not all of the same species whether derived from milk or from pathological conditions in man.

The immune serum of the goat treated with the streptococcus derived from a case of scarlet fever, agglutinated the streptococci insolated from milk in a lower degree than the culture used for purposes of immunization. The immune serum of the goat treated with the streptococcus isolated from cases of contagious mammitis acted equally well upon the streptococci isolated from the milk of cows, and those isolated from pathological conditions in man.

The most marked differences in the degree of agglutination of the immune sera upon different cultures of streptococci is seen in cultures that produce long chains and those which produce short chains. All the cultures producing long chains were agglutinated in relatively high dilutions, while the cultures producing short chains were uniformly agglutinated in low dilutions only. Whether these differences in the relative agglutinability are due merely to differences in vital activity of the organisms, or whether it is due to differences in receptor apparatus of the two groups of organisms, can not be stated at present.

All efforts to procure a culture of streptococcus derived from strangles in horses failed, cultures of streptococci derived from cases of rheumatism, erysipelas and septicaemia were also studied, and the results were identical with those obtained with the scarlet fever culture. The results obtained in agglutination tests with streptococci derived from different pathological conditions in man by Aronson and others, indicate, however, that no differences can be certainly noted, so that they have concluded that the streptococci derived from these different sources are the same. Some investigators also claim that the streptococcus derived from strangles in horses is the same as that found in man, judging by its behavior towards immune sera. So far none of the milk streptococci had been compared in this manner with the other streptococci, and it is interesting to note that here likewise no definite differences can be distinguished.

SUMMARY OF THE RESULTS OBTAINED:

1. In the samples of milk drawn directly from the udder, the number of bacteria found ranged from none (in 32 per cent. of the samples) to 93,100 per cubic centimeter. The prevailing bacteria found in these samples are Streptococcus, Staphylococcus and Bacillus pseudodiphtheria. Other bacteria were also found in limited numbers in some of the samples and these were no doubt

derived from the orifice of the teat, the hands of the milker, the hair of the cow, or the air of the stables and laboratory.

2. The bacteria which gain access to milk in modern dairies during the manipulations of the milk in milking, straining, and cooling, are evidently derived from several sources, as the air of stables and milkhouses, the hair of the animal, and from the different milk utensils, the latter being by far the most fruitful source of the bacteria.

The bacteria gaining access to milk in the ordinary manipulations in modern dairies are largely air, water, and soil organisms, as shown by the preponderance of organisms of the type of putrefactive bacteria. The occurrence of the group of lactic bacteria in such milk was found to be quite insignificant, though it is probable that these organisms find in milk a more suitable field of activity than do the putrefactive bacteria and, hence, usually exceed them in numbers by the time the milk reaches the consumer.

The occurrence of Bacillus coli and Bacillus alkaligenes in these samples of milk indicates contamination with manure, though these bacteria may gain access to the milk in an indirect manner by being carried in the air, or by flies.

3. Cells can be demonstrated in the milk of practically all cows, and hence the number of these cells present in milk becomes a matter of importance. It is believed that the occurrence of ten cells per field of the 1-12 immersion lens indicates the presence of pus in milk, especially if the cells occur in masses. The presence of pus in milk always denotes an inflammatory reaction within the udder, from the fact that the pus is always associated with pyogenic organisms.

4. Streptococci were found in nearly all the samples of milk, derived from cows which showed the presence of pus. These bacteria are usually the cause of catarrhal mammitis, and are always encountered in the contagious mammitis.

Streptococci and pus cells were also encountered in samples of milk derived from cows in which no inflammation of the udder could be discovered. This occurrence is probably due to the fact that the disease was not very active or that it had persisted for a considerable time and become chronic.

5. Comparative studies of the different cultures of streptococci isolated from milk revealed no marked morphologic or biologic differences. Neither could these cultures be differentiated by this means from cultures of streptococci isolated from pathological conditions in man.

The immunization of several goats with cultures of streptococci isolated from milk and from pathological conditions in man, failed to show any definite differences in the agglutinating power of the

different sera against the various cultures of streptococci, whether these were of human or of bovine origin.

## CONCLUSIONS.

1. The results obtained with samples of milk drawn in sterile test tubes directly from the udder of individual cows, show that about one-third of the samples are free from bacteria, while only about 10 per cent. of the samples contain large numbers (over 5,000 per cubic centimeter) of bacteria.

2. The prevailing bacteria, found in the samples of milk showing the presence of large numbers of bacteria, are the pyogenic organisms, and these large numbers of bacteria are usually associated with inflammatory reactions in the udder.

3. The bacteria which gain access to the milk after it leaves the udder are derived in part from the hair of the cow, the hands and clothing of the milker, and the air of the stables; but the greater proportion of the bacteria gaining access to the milk during milking and the subsequent straining and cooling of the milk, are derived from the utensils and apparatus employed for this purpose.

4. The principal varieties of bacteria gaining access to the milk after it leaves the udder are common air, water, and soil organisms; organisms of putrefaction; and the lactic acid bacteria. Of these, the putrefactive organisms were found quite frequently.

5. The sterilization of all the milk utensils, straining and bottling apparatus, and the washing of the milker's hands and the cow's udders, are most important measures in limiting the number of bacteria in milk.

6. The occurrence of more than ten cells per field of the 1-12 immersion lens usually indicates the presence of pus in the milk of an individual cow. In milk containing more than ten pus cells per field, it is usually possible to demonstrate also considerable numbers of the organisms of suppuration, more especially of streptococci.

7. The occurrence of considerable numbers of streptococci in a cow's milk indicates that some inflammatory reaction is going on in the udder, and comparative studies of milk drawn from each quarter of the udder show that the disease is frequently confined to one quarter entirely or in large part, while the remainder of the udder is free from the disease.

8. Because of the danger of conveying the infection to other cows, it is advisable to isolate infected cows from the herd and sterilize their milk until such time as detailed examination shows their complete recovery. The milk of these cows, and the fore-milk of any cow should not be thrown upon the stable floor, but

should always be collected in a special vessel and subsequently sterilized and used to feed pigs or young cattle.

9. Cows suffering from contagious mammitis should be removed from the dairy at once and prepared for the butcher since, so far, no satisfactory treatment has been discovered for this disease.

10. It is probable that the peculiar yellow color of the milk in contagious mammitis is due in large part to the pigment-producing properties of the streptococcus producing the disease. The yellow color of the milk in this disease may also be due in part to the presence of small quantities of the coloring matter of the blood, owing to the destructive action of the poison formed by the streptococci upon red blood corpuscles.

11. Careful study of the streptococci isolated from the milk in different dairies has revealed no definite difference between these organisms and the streptococci encountered in pathological conditions in man, hence, it is probable that they all belong to the same species.

12. The conditions in a dairy which appear to be necessary to produce a milk which is satisfactory in respect to its bacterial content, may be stated as follows:

a. The selection of cows free from inflammatory disease of the udder.

b. Housing the cows in clean, well-ventilated stables, with non-dust producing bedding.

c. The systematic, daily cleaning of the cows with curry-comb and brush, and the washing of the udder and flanks with clean warm water a short time before each milking.

d. The careful regulation of the diet and physical condition of the cows so as to maintain their health.

e. The selection of efficient milkers and attendants who will faithfully carry out the minutest details of the regulations necessary to produce pure milk.

f. The provision of flowing water, soap and towels to permit the milkers to wash and scrub their hands after milking each cow.

g. The provision of freshly laundered cotton slips and caps to be worn by the milker over his regular clothing.

h. The sterilization by means of steam of the various milking, straining, cooling and bottling apparatus before each milking.

i. The rapid cooling and bottling of the milk so as to remove it permanently from contact with air at the earliest possible moment. The milk should be cooled to about 40 degrees F. before it is bottled.

j. The transportation and storage of milk in such a manner as to prevent its temperature rising much above the point to which it has been cooled during transportation, so that it may reach the consumer without undergoing any alteration.

It is believed that all of these measures are necessary in order to produce a satisfactory milk, though it is evident that some of them are of greater importance than others. Those that are of primary importance with regard to the bacterial content of the milk are: a, e, h, i and j. The special value of these five measures does not, however, indicate that the other five are of little value since the neglect of any one of them may reduce the value of all the measures.

## GLOSSARY.

Agglutination, a term applied to the massing together of bacteria when they are suspended in blood serum.

Bacillus (plural bacilli), a rod-shaped organism.

Bacillus pseudodiphtheria, an organism which has many of the characters of the diphtheria bacillus, but is usually without pathogenic effect.

Bacteria (singular bacterium), the smallest forms of vegetable organisms, composed of single cells.

Centrifuging, the separation of suspended particles in fluids by means of a centrifugal machine.

Colony, an aggregation of bacteria that have developed from a single organism in a solid medium.

Cubic centimeter, a volume equal to 1-960 part of a quart.

Immune, that state of an organism in which it is tolerant of large doses of foreign substances which would otherwise be detrimental.

Immunization, the process of rendering an animal tolerant to foreign substances by the introduction of increasing doses.

Incubator, a double-walled chamber which is maintained at a uniform temperature, in which bacterial cultures are grown.

Inoculation, the act of transferring bacteria from one medium to another, or from a medium into the tissues of an animal.

Leucocytes, the white or colorless corpuscles of the blood.

Mammitis, inflammation of the udder.

Micrococcus (plural micrococci), bacteria that are spherical in shape.

Micro-organism, any microscopic form of life, a term commonly applied to bacteria.

Organism, any form of life that maintains an independent existence.

Pathogenic, having the power of producing disease.

Petri plate, a small flat glass dish with closely fitting cover.

Plating, the act of distributing mixtures of bacteria and melted agar or gelatin in special covered glass dishes (Petri plates).

Pus cells, dead leucocytes and other cells contained in pus.

Staphylococcus (plural staphylococci), spherical bacteria that

group themselves in irregular bunches; the bacteria which are concerned in the production of inflammation, abscesses, carbuncles, etc.

Sterile, free from all living organisms.

Streptococcus (plural streptococci), spherical bacteria that group themselves in chains or strands; the bacteria which are concerned in the production of spreading inflammations, erysipelas, septicaemia, etc.